

STUDIES ON GRANULOMA INGUINALE

VIII. SEROLOGICAL REACTIVITY OF SERA FROM PATIENTS WITH CARCINOMA OF PENIS WHEN TESTED WITH *DONOVANIA* ANTIGENS*

BY

J. GOLDBERG AND H. ANNAMUNTHODO

Department of Microbiology and Public Health, Chicago Medical School, and Department of Surgery, University of the West Indies

An association between granuloma inguinale and genital carcinomas has long been suspected (McKay and Bunch, 1952; Alexander and Shields, 1953; Saltzstein, Woodruff, and Novak, 1956) for the following reasons:

- (1) The higher incidence of genital carcinomas in areas where granuloma inguinale is endemic, as compared to areas in which granuloma inguinale is not found to any appreciable extent.
- (2) The occasional occurrence of carcinoma within depigmented areas of healed granuloma inguinale lesions.
- (3) The concurrence of carcinoma and granuloma inguinale in the same lesion. While this view has not been accepted by investigators in all parts of the world (Rajam and Rangiah, 1954), it is firmly held by the majority of clinicians in Jamaica (Stewart, 1959; Annamunthodo, 1959, 1961).

To test the supposed association between genital carcinoma and granuloma inguinale, it was thought appropriate to test the sera of patients with proven genital carcinoma to determine if any of these sera contained antibodies directed against *Donovania granulomatis* antigens. Previous work (Goldberg, Weaver, Packer, and Simpson, 1953) has shown that this technique is a moderately sensitive but highly specific test for the diagnosis of granuloma inguinale. At the same time, sera from control patients, matched to each carcinoma patient as to age, sex, and socio-economic grouping, would be secured and tested in an identical manner, the

rationale being that, if there was an association between carcinoma of the genitalia and granuloma inguinale, sera from patients with carcinomas should show antibodies directed against the aetiological agent of granuloma inguinale. Further, this positive association should occur more often in this group than in a group of patients with diseases other than genital carcinomata, excepting granuloma inguinale cases.

In this paper we report the results of serological studies on sera from 62 patients with histologically proven carcinoma of the penis and their matched controls. The results of serological studies on patients with carcinoma of the cervix or vulva will be presented at a later date.

Methods

All the sera from patients with carcinoma of the penis and their controls were collected at the University Hospital of the West Indies, Kingston, Jamaica. Specimens of blood were secured by venepuncture and the separated sera stored at -20°C . until tested at the laboratory of the senior author in Chicago, Illinois. A 100 per cent. complement-fixation test with all reagents in 0.2 ml. volumes was used. Anticomplementary sera were not included in the tabulations. In the tests three antigen preparations were used:

- (1) *Anderson Bacterial Antigen* prepared from the prototype strain of *Donovania granulomatis*. This antigen is essentially a washed suspension of the bacterium grown in liquid medium. Previous work had shown that this antigen was not anticomplementary at any dilution and was highly specific for granuloma inguinale (Goldberg and others, 1953).
- (2) *Anderson Filtrate Antigen*: a protein-free filtrate of the Anderson growth fluid medium which

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contains a carbohydrate moiety which is serologically active and probably represents capsular material from the bacterium.

- (3) *Franklin Bacterial Antigen*: This is a strain of *Donovania* isolated by the senior author from a case of granuloma inguinale. It was prepared in a manner identical to that of the Anderson bacterial antigen.

Histopathology

The histopathology of the biopsy material taken from each case of carcinoma of the penis was studied independently. In most cases the diagnosis of squamous cell carcinoma presented no great difficulty; the usual criteria of unequivocal, gross cellular atypia, many and abnormal mitotic figures, large and multiple nucleoli, nuclear hyperplasia and hyperchromia, and individual cell keratinization were present. Occasionally, the diagnosis was made on a lymph node metastasis. Great care was taken to exclude possible cases of pseudo-epitheliomatous hyperplasia.

The histological diagnosis of granuloma inguinale and its differentiation from carcinoma was made on the basis of a dense dermal infiltration with micro-abscesses, plasma cells, and macrophages containing the capsulated intracytoplasmic Donovan bodies. Marked acanthosis is often present in granuloma inguinale, but individual cell keratinization, nuclear hyperplasia, and hyperchromasia are absent.

Results

These are outlined in Table I (opposite); the control sera gave uniformly negative results with all three of the antigens used, while nine of the sera from patients with carcinoma of the penis were positive with one or more of the test antigens. Three of these sera were positive with all three of the antigens, one with the Anderson filtrate antigen only, two with the Anderson filtrate and Franklin

bacterial antigens, three with the Franklin bacterial antigen only, and one with the Anderson and Franklin bacterial antigens but not with the Anderson filtrate antigen. In order to be considered as positive a 4+ fixation of complement must have occurred in at least one dilution of serum. Two additional sera gave weak reactions with the Franklin antigen only but were not considered as positive because of the lack of 4+ fixation in any dilution. This type of serological variability is similar to the reactions observed when known granuloma inguinale sera are tested; presumably because of the antigenic variation in strains of *Donovania* (Goldberg, 1954).

When the reactions of the sera are classified according to the age of the patient (Table II), it may be noted that positive reactions were given by two of three sera of 20 to 29-year-old patients, three of ten sera from 30 to 39-year-old patients; two of fourteen sera from 40 to 49-year-old patients; one of seven sera from 50 to 59-year-old patients, and none of twelve sera from 60 to 69-year-old patients.

The interpretation of these results is difficult until certain pertinent questions are answered:

- (1) Is this pattern of association of genital carcinoma with granuloma inguinale unique to this disease or do other venereal diseases exhibit the same association?
- (2) May not the correlation of young age group with a high percentage of positive serological reactions for granuloma inguinale indicate that these cases were really cases of granuloma inguinale rather than carcinoma of the penis, especially since both may show histological and clinical similarities?
- (3) If there is an association between carcinoma of the penis and granuloma inguinale should one not expect to find more than 15 per cent.

TABLE II

SEROLOGICAL REACTIVITY OF SERA FROM PATIENTS WITH CARCINOMA OF THE PENIS AND CONTROLS, BY AGE GROUP

Age Group (yrs)		Granuloma Inguinale		Matched Controls		Lygranum		Matched Controls	
		No. +	No. in Group	No. +	No. in Group	No. +	No. in Group	No. +	No. in Group
20-29		1	3*	0	3	1	3	0	3
30-39		3	10	0	10	4	10	2	10
40-49		2	14	0	14	8	14	4	13
50-59		1	17	0	17	5	17	2	17
60-69		0	12	0	12	5	12	4	12
70-79		2	5	0	5	0	4	2	5
80+		0	1	0	1	1	1	0	1
Total	No.	9	62	0	62	24	61	14	61
	Per cent.	14.5		0.0		39.3		22.95	

TABLE I

SEROLOGICAL RESULTS WITH SERA, FROM 62 PATIENTS WITH CARCINOMA OF PENIS AND MATCHED CONTROLS

Patient No.	Age (yrs)	CARCINOMA OF PENIS				MATCHED CONTROLS			
		Anderson Bacterial	Anderson Filtrate	Franklin Bacterial	Lygranum	Anderson Bacterial	Anderson Filtrate	Franklin Bacterial	Lygranum
1	56	—	—	—	—	—	—	—	—
2	68	—	—	—	—	—	—	—	10*
3	82	—	—	—	—	—	—	—	—
4	49	—	—	—	10	—	—	—	—
5	51	—	—	—	5	—	—	—	—
6	33	—	—	—	—	—	—	—	5
7	53	—	10	10	10	—	—	—	—
8	76	—	10	5	—	—	—	—	—
9	57	—	—	—	—	—	—	—	—
10	50	—	—	—	—	—	—	—	—
11	26	—	—	—	—	—	—	—	—
12	50	—	—	—	—	—	—	—	—
13	52	—	—	—	40	—	—	—	—
14	50	—	—	—	—	—	—	—	5
15	58	—	—	—	10	—	—	—	—
16	67	—	—	—	—	—	—	—	—
17	45	—	—	—	—	—	—	—	—
18	35	—	—	—	—	—	—	—	—
19	62	—	—	—	—	—	—	—	—
20	65	—	—	—	—	—	—	—	40+
21	69	—	—	—	—	—	—	—	—
22	34	—	—	—	—	—	—	—	—
23	52	—	—	—	—	—	—	—	—
24	40	—	—	±	—	—	—	—	—
25	50	—	—	—	—	—	—	—	—
26	40	—	—	—	—	—	—	—	—
27	57	—	—	—	—	—	—	—	—
28	62	—	—	—	—	—	—	—	—
29	33	80+	80	10	10	—	—	—	—
30	50	—	—	—	—	—	—	—	—
31	38	—	—	—	—	—	—	—	—
32	27	—	—	—	—	—	—	—	—
33	49	5	5	10	—	—	—	—	10
34	29	—	—	—	40	—	—	—	—
35	49	—	—	—	—	—	—	—	10
36	60	—	—	—	20	—	—	—	—
37	36	—	—	—	40+	—	—	—	—
38	64	—	—	—	40	—	—	—	—
39	49	5	—	10	40	—	—	—	—
40	78	—	—	—	—	—	—	—	—
41	42	—	—	—	40	—	—	—	10
42	60	—	—	—	10	—	—	—	—
43	61	—	—	—	—	—	—	—	40
44	38	—	—	—	40+	—	—	—	10
45	41	—	—	—	10	—	—	—	I.S.
46	59	—	—	—	80+	—	—	—	—
47	45	—	—	—	10	—	—	—	—
48	72	—	—	—	—	—	—	—	40
49	45	—	—	—	—	—	—	—	—
50	58	—	—	—	—	—	—	—	40+
51	31	—	80	—	40+	—	—	—	—
52	76	I.S.	—	20	—	—	—	—	—
53	30	—	—	—	—	—	—	—	—
54	30	5	5	10	—	—	—	—	—
55	47	—	—	—	20	—	—	—	—
56	63	—	—	—	40	—	—	—	10
57	58	—	—	—	—	—	—	—	—
58	45	—	—	—	5	—	—	—	10
59	72	—	—	—	5	—	—	—	10
60	55	—	—	—	—	—	—	—	—
61	66	—	—	—	10	—	—	—	—
62	48	—	—	—	40+	—	—	—	—

* Titres expressed as reciprocals of highest dilution showing 3 or 4 plus fixation.

of the cases of carcinoma of the penis showing positive reactions with Donovanian antigens?

In an attempt to answer the first question, all 62 CaP sera were tested with Lygranum, an antigen prepared from the lymphogranuloma venereum (LGV) virus which is used routinely in the serological

diagnosis of LGV. The CaP control sera were also tested.

The results obtained are outlined in Tables I and II, which show that proportionally more of the CaP sera reacted with the Lygranum antigen than did the control sera. 24 of the CaP sera were reactive,

compared with 14 of 61 control sera. Moreover, if the titres of the reactions are plotted (Table III) those of the CaP sera are higher than those of the control sera. For example, 54 per cent. of the positive results observed with CaP sera were 1:20 or higher, while only 29 per cent. of the control sera were in this range. However, this apparent association is only suggestive for a χ^2 analysis, using Yates' correction factor, indicated that this variation was not statistically significant ($\chi^2_{(1)} = 3.48$; $P > 0.05$).

TABLE III
TITRES OF CaP SERA AND CONTROL SERA WITH
LYGRAM ANTI-GEN

Sera	Negative	Positive		
		1:5-10	1:20-40	1:40+
CaP	37	11	8	5
Matched Controls	47	10	2	2

There does not seem to be an association between granuloma inguinale and lymphogranuloma venereum, for in the CaP group of patients with positive serological tests for granuloma inguinale four out of nine (44 per cent.) were positive with LGV antigen, and twenty out of 53 (38 per cent.) of the CaP sera giving negative results with granuloma inguinale antigen were positive with LGV antigen (Table IV).

Approximately the same percentage were positive for LGV regardless of their granuloma inguinale serology.

TABLE IV
RESULTS OF SEROLOGICAL TESTS FOR GRANULOMA
AND LYMPHOGRANULOMA VENEREUM IN SERA FROM
62 PATIENTS WITH CARCINOMA OF THE PENIS

Granuloma Inguinale	Lymphogranuloma	No. of Patients
Positive	Positive	4
Positive	Negative	5
Negative	Positive	20
Negative	Negative	33
Total		62

It is interesting to note that the percentage of positive LGV serology with CaP sera is similar to that which we have previously observed in another group of granuloma inguinale patients — of 88 sera from proven cases of granuloma inguinale which were serologically positive for granuloma inguinale, forty (45.4 per cent.) were positive when tested with Lygram antigen.

The second point, which questions the validity of the diagnosis, is difficult to disprove objectively.

It may be pointed out that the clinicians and pathologists at the University hospital have great experience in this field, which has shown that, in numerous cases in which the diagnosis was in doubt (in so far as carcinoma was concerned) and surgery was not performed, resultant metastases were later found. In addition, in those cases in which the histopathological diagnosis was in doubt on the basis of a biopsy, when the surgeon proceeded with amputation on the basis of clinical features, pathological examination of the penis in every case to date revealed unequivocal invasive carcinoma.

Data pertinent to the third point are sparse. The low percentage of positive reactions for granuloma inguinale in older individuals may be due to lowering of antibody titre after spontaneous regression or to the use of antibiotic therapy. There are few data on the duration of antibody titres. We have tested eight sera from proven cases of granuloma inguinale 2 years after successful chemotherapy, and found that half of them had reverted to negative, although each was sero-positive before therapy. What the results would be after a greater length of time is not known.

Discussion

Differentiating between causal and non-causal associations is a difficult undertaking, especially in relation to diseases in which the aetiological agents are uncertain. We wish to emphasize that, at this time, we are not suggesting an aetiological relationship between granuloma inguinale and carcinoma of the penis. What we do wish to present is additional laboratory evidence which strengthens the clinical impression of an association between carcinoma of the penis and granuloma inguinale, at least as observed in Jamaica.

The fact that sera of patients with carcinoma of the penis reacted with antigen derived from *Donovania granulomatis* is suggestive of an association, especially since control sera were completely negative. It may well be, if the transmission of granuloma inguinale occurs *via* faecal contamination (Goldberg, 1964), that the association between carcinoma of the penis and granuloma inguinale may be due to a common third factor, as, for example, that of poor hygiene. This has been suggested by Schrek and Lenowitz (1947) who reported that, in a series of patients with carcinoma of the penis, positive correlations were obtained for the factors carcinoma of the penis, syphilis, gonorrhoea, and coloured race (presuming low socio-economic status).

Other interpretations are possible, and we feel that the observed association between carcinoma of the penis and granuloma inguinale is strong enough

to warrant further experimentation on the oncogenic activity of *Donovania* products.

Summary

Sera from 62 histologically proven cases of squamous cell carcinoma of the penis and matched controls were tested with *Donovania granulomatis* antigens. Nine of the CaP sera and none of the control sera reacted in a positive manner. When the same sera were tested with lymphogranuloma venereum antigen, 24 of the CaP sera and fourteen of the control sera showed positive reactions. This difference in reactivity to LGV antigen was not statistically significant.

It would appear that the clinical impression of an association between CaP and granuloma inguinale is strengthened by our results.

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Des études faites au sujet du granulome inguinal

VIII. La réaction sérique des malades souffrant du cancer de la verge et les antigènes de Donovan

RÉSUMÉ

Le sérum de 62 cas de cancer squameux de la verge prouvés histologiquement et celui des contrôles ont été testés avec les antigènes du *Donovania granulomatis*. Neuf des sérums des cancers de la verge et aucun des sérums des contrôles n'ont réagi d'une manière positive. Quand les mêmes sérums ont été testés avec l'antigène du lymphogranuloma venereum, vingt quatre des sérums des cancers de la verge et quatorze sérums des contrôles ont donné une réaction positive. La différence de réactions à l'antigène du lymphogranuloma venereum n'était pas significative au point de vue statistique.

Il semblerait que l'impression clinique d'une association entre le cancer de la verge et le granulome inguinal est renforcée par nos résultats.